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THE EXPULSION OF ASCOSPORES FROM THE PERITHECIA OF THE CHESTNUT BLIGHT FUNGUS, ENDOTHIA PARASITICA (MURR.) AND.

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INTRODUCTION

General historical consideration of ascospore expulsion from perithecia.—The scope of the present article will not permit an exhaustive treatment of the subject of spore expulsion, but it seems desirable to point out at the beginning some of the more noteworthy observations, and especially those which apply to pyrenomycetous forms.

Forcible expulsion of spores as a means of aiding in their dissemination takes place in many fungi and is accomplished in a variety of ways. The projection of the entire sporangia as in *Pilobolus crystallinus*, or of the single spores as in species of Empusa and Entomophthora, are well known illustrations. Cases of forcible expulsion of spores by Ascomycetes have long been known, and more recently similar phenomena have been demonstrated for Hymenomycetes (4, 7) and Uredinales (5, 7). Although the height of projection is never great it is incomparably greater in the ascomycetes (few mm. to 15 cm.) than in the basidiomycetes, as has been pointed out by Falck (8).

Directing our attention to Ascomycetes, the simultaneous ejection of the spores from a considerable number of asci giving rise to "puffing" may be noted. This phenomenon has been known since the time of Persoon (1798) and Desmazieres for the larger Discomycetes, and according to De Bary (6) simultaneous ejection of the spores from the

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ascus occurs in nearly all species of this class. The forcible expulsion of spores from perithecia was not observed however until a later time. Several of the noteworthy observations may be mentioned. Tulasne (20) observed the ejection of the spores from the perithecia of Claviceps in 1861; this was confirmed by De Bary (6) and the same phenomena noted for Cordyceps. The details of the process of spore expulsion were studied by Pringsheim (13) for *Sphaeria scirpi* in 1858; by Woronin (24) for *Sphaeria lemaneae* in 1869; by Wolff (23) for Erysiphe in 1875; by Zopf (25) for Sordaria species in 1880; while De Bary in 1884, in his discussion of the expulsion of spores, claims that the process is common in the Pyrenomycetes. It may be pointed out in this connection that ascospores are not infrequently set free from the perithecium by the mucilaginous swelling of the asci and are forced out embedded in mucilage in drop-like aggregations or in the forms of tendrils or spore-horns instead of being set free by the explosion of the asci. Pyrenomycetes showing the two types of spore expulsion have been designated as *active* and *inactive*. In the active forms, according to De Bary, the entire spore content of the ascus may be ejected *simultaneously* as first described by Zopf (25) for Sordaria, or the spores may be expelled in *succession* as was first shown by Pringsheim (13) for *Sphaeria scirpi*. In connection with this claim the more recent view of Falck (8) should be presented: "According to my investigations conducted on species from all classes, I can state it as a rule that in all active Ascomycetes the spores are shot out singly, that is, one spore after the other at regular intervals. The intervals are mostly so short that the ejection appears to the eye as single. In proper microscopic preparations in which the ejection is often retarded, one can follow directly how one spore after the other is emptied from the ascus."

According to all published observations, to be referred to later, the expulsion of the spores from the ascus in Endothia is *simultaneous*.

The forcible expulsion of the ascospores of the black rot fungus, *Guignardia bidwellii*, was noted by Scribner and Viala (18) and the following main facts established:

1. The ascus swells up to double length and is set free from the perithecium through the ostiole or by the rupture of the wall. 2. When the asci have escaped they expel the contained spores one at a time. 3. The maximum height of projection was 4 cm. 4. A temperature of 20°–30° C. was necessary for expulsion.

The importance of the ascus discharge in the dissemination of the black rot of the grape is brought out by Reddick (17) who has confirmed the observations of Scribner and Viala, and Wallace (21) has made a similar contribution in his study of the life-history of the apple scab fungus, *Venturia inaequalis*. Perhaps the most notable recent work is that of Falck (8) on the air infection of ergot, and the dissemination of infectious diseases of plants by means of convection currents ("Temperatur-strömungen"). In the course of this work Falck points out the effectiveness of convection currents in the dissemination of ascomycetous spores.

Expulsion of ascospores by the chestnut blight fungus.—The earlier reports on the chestnut blight fungus make no mention of the way in which the ascospores are set free from the perithecia. Although the first studies appeared in 1906 it was not until the summer of 1911 that Rankin (14) observed the forcible expulsion of the ascospores. Later (15) the same writer says: "Under moist conditions the ascospores are shot forcibly out in the air where they can be caught up by the wind and carried for a considerable distance. The speaker found the ascospores being shot from mature pustules during every rainy period last summer." Considering the fact that forcible expulsion of ascospores by many pyrenomycetous fungi was a fairly well-known phenomenon, as has been pointed out in the previous pages, it seems strange that this important observation did not come earlier. It was this which served as the impetus for investigations along new lines on the dissemination of the fungus.

Additional observations and experiments on the expulsion of the ascospores made at the Field Laboratory of the Pennsylvania Chestnut Tree Blight Commission during the summer of 1912 are summarized by Anderson (1) and presented in detail by Anderson and Babcock (2). The most important points brought out by these investigations were as follows:

1. Ascospore expulsion occurs only during and for a short period after rains or as long as the bark remains wet. 2. The maximum distance to which ascospores were expelled vertically was 22 mm., while the maximum horizontal distance was 89 mm. 3. The rate of ejection under favorable conditions was an ascus explosion about every two seconds or an average of 4.06 spores per second. 4. Bark bearing perithecia and kept moistened continued to expel ascospores for 25 days.

The way in which the ascospore expulsion occurs was pointed out by Rankin (16). According to his observations the asci are set free and rise in succession to the ostiole, where the explosion takes place, eight spores, or the entire contents of an ascus, being liberated at each pulsation. These observations were confirmed by Anderson (3) and in connection with the study of the morphology of the ascus, the mechanics of the spore discharge was briefly considered.

The senior author first pointed out the relation of temperature to the expulsion of ascospores (9), showing that moisture and the proper temperature are essential to the proper activity of the process. This relation was again emphasized (10) and the bulletin referred to contains the first published illustration of a characteristic spore print on an object slide. The first data on relation of temperature to ascospore expulsion were obtained from experiments conducted in constant temperature rooms, and these were substantiated later by tests carried out under field conditions (11). There was no expulsion of ascospores from Nov. 26, 1912, until the rain of March 21, 1913, although there were 21 warm winter rains during this period with maximum temperatures during or immediately following the rain of 35–60 degrees Fahr.

The length of time during which ascospore expulsion occurred following various warm rains was given special attention in some recent studies on air and wind dissemination of the chestnut blight fungus (12). It was found to depend upon the weather conditions following the cessation of a rain, and varied from 45 minutes to a maximum of 13 hours.

Our work on the relation of temperature to ascospore expulsion has been summarized by the junior author (22).

Two facts established by the researches of Falck have a very important bearing on the dissemination of the spores of Endothia. First, he has shown that "Temperatur-strömungen" alone suffice for spore dissemination of Claviceps; and for this reason fields protected by woods from strong winds and remaining moist longer than free lying fields are more infected. Second, he points out the effect of evaporation in the production of convection currents. It is certainly worthy of note that the ascospores of Endothia are being expelled under natural conditions in the field at a time when evaporation from the bark is favorable to the creation of convection currents (12). For this reason we chose to speak of the "air and wind dissemination"

of the spores, since an evident wind is not necessary to scatter the spores, but only helps to secure a wider dissemination.

METHOD

A study of various phases of ascospore expulsion under artificial conditions has been carried out during the past year. A series of nine main experiments covering practically the entire year of 1913 was conducted. Specimens of excellent quality showing mature perithecial stromata were obtained at Emilie, Bucks County, Pa., and fresh material was always collected for each test. Great care was taken not to let the specimens dry out previous to starting the experiments.

In conducting the tests, small pieces of bark of approximately 3.5 × 1.5 inches were placed on two layers of blotting-paper in shallow granite trays, 8 × 12 inches. Six pieces of bark were usually used for each tray and in selecting the specimens care was taken to secure pieces with a uniform distribution of perithecial stromata. Control specimens were always used and whenever possible they were taken from the other half of pieces used for the test. If this was not possible pieces of bark of uniform character were selected. Slides were supported and fastened over the bark by the following method: Two pieces of match sticks of the same length as the width of the slides, were dipped in melted wax (beeswax and resin) and one placed across each end of a slide. The wax was allowed to harden and the sticks were held securely. When placed on the bark the slides were supported about 2–3 mm. above the perithecial necks and thus sufficiently near to catch the ascospores on their under surfaces whenever expulsion occurred. In order to support the slides over the same material each day, four to six pins were stuck into the bark at the sides and ends of the slides. Each time before moistening the specimens the slides were removed and water of the same temperature as the room or incubator chamber was sprayed on the surface of the specimens and the blotting paper was also thoroughly drenched. Records were taken once a day, that is, the individual spots, each of which represents an active ostiole, were counted and their density noted. Not less than twelve specimens were used for each experiment, except in one or two cases with minor tests, and a like number was used for the control. Complete temperature records were obtained for each experiment by the use of Friez thermographs.

The Relation of Temperature to Ascospore Expulsion

The relation of temperature to the forcible expulsion of ascospores from perithecia appears to have been passed over with only brief consideration. In a few investigations the relation of this process to proper temperature conditions has been mentioned. Scribner and Viala (18) stated that a temperature of 20–30 degrees C. was necessary for the forcible ejection of the spores of the black rot fungus, but gave no details of experiments upon which this assertion was based. Falck (8) in a discussion of the dissemination of Claviceps spores by convection currents, brings out the point that this phenomenon is least pronounced at cellar temperatures, and attributes this entirely to the lack of "Temperatur-strömungen," but unfortunately the activity of the process of expulsion was not taken into consdieration. It seems probable from our results that spore expulsion would be much less active at cellar temperatures. At another point he makes the statement that temperature has a pronounced effect on the rapidity of spore expulsion, increasing with rise in temperature, but no record is given of the temperatures at which observations were made.

No exhaustive attempt has been made to bring together the literature dealing with the relation of temperature to ascospore expulsion in general, but it can be definitely stated that no previous work bearing on the relation of temperature to ascospore expulsion in the chestnut blight fungus has been published.

In studying the relation of temperature to expulsion of ascospores of the blight fungus nine tests were carried out covering a range of temperature from 36.5° F. to 100° F. No expulsion was obtained at the lowest temperature employed but with each increase in temperature came an increase in expulsion until the optimum, 70°–80° F., was reached, beyond which expulsion gradually lessened. At 100° F. it had practically ceased. The nine tests were as follows:

(*a*) *Temperature 36.5°–40° F. Average 38.6° F.* The specimens were collected at Emilie, Bucks County, Pa., on Dec. 22, 1912, prepared by the method described, and the experiment started two days later with 21 pieces for the test and 23 for the control. The specimens were kept at this temperature in a cold room for 12 days where there was absolutely no expulsion. They were then moved to the laboratory where for the first three days they remained inactive, but after that time there was a gradual increase until at the end of two weeks all

but one specimen had expelled ascospores. The expulsion was on the whole rather moderate. The control kept at laboratory temperature, about 72° F., shot abundantly.

(*b*) *Temperature 52.8°–54.9° F. Average 53.8° F.* The specimens were collected at Oxford, Chester Co., Pa., Dec. 30, 1912, prepared as described above and the experiment started Jan. 6, 1913 with 20 specimens for the experiment proper and 19 for the control. The trays were kept in a cold room for two weeks at an average temperature of 53.8° F. Shooting was very light, as may be noted from the following summary:

TABLE I

NUMBER OF SPECIMENS SHOWING EXPULSION AT 53.8° F.

No. of days shooting occurred	0	1	2	3	4
No. of specimens	6	5	4	2	3

It may be seen from this tabulation that 6 specimens showed no expulsion whatever for the entire period of two weeks, while the maximum amount was given by three specimens which gave expulsion on four different days. After the first two days only single spots were obtained from any specimens. At the end of two weeks they were moved to laboratory temperature, about 72° F. for 12 days where expulsion occurred from all but one specimen. Shooting was moderate. The trays were again moved to the cold room, this time for 13 days, at an average temperature of 54.9° F. For the first two days expulsion continued as at laboratory temperature but after this time it was very light and corresponded to the first two weeks in the cold room. The control at the laboratory temperature, average 72° F., shot abundantly.

(*c*) *Temperature 54°–59° F. Average 56.25° F.* The specimens were collected at Emilie, Bucks Co., Pa., Nov. 19, 1913, and prepared according to the regular methods, but the experiment was not started until Dec. 3, 1913. This delay was caused for the reason that the cold room had not been adjusted to the proper temperature. The trays for the experiment were put in the cold room and those for the control in the laboratory Nov. 20, 1913 and the blotting paper in the trays moistened each day to prevent the specimens from drying out. Dec. 3, 1913, the bark was wet and the test started with 12 specimens for the experiment and 12 for the control. The trays were kept in the cold room for 10 days at an average temperature of *56.25° F.*

Expulsion was light but all the specimens shot more or less. The control specimens at laboratory temperature, 70.8° F., gave an abundant spore expulsion.

(*d*) *Temperature 59°–65.5° F. Average 62.5° F.* The specimens were collected at Emilie, Bucks County, Pa., Feb. 6, 1913, and prepared by the usual method. From the time of collection until February 13 the specimens were kept moist. The bark was wet and the test started on this date but the trays were kept for two days at laboratory temperature to make certain that the material was in the proper stage of development for expulsion. On February 15, 1913, two trays were put in the cold room and two kept in the laboratory for a control. 12 specimens were used for the experiment proper, and 12 for the control. The test specimens were kept in the cold room at an average temperature of 62.5° F. for 17 days, during which time expulsion was light. All of the specimens showed some active ostioles. The control specimens shot abundantly. At the end of the 17 days, on March 4, 1913, the two cold room trays were put in a room held at a temperature of 24° F. for one week. The bark was of course frozen solid during this time. At the expiration of the week, they were again removed to the first cold room and observed for 19 days, or until March 31. The first day after removal from the 24° F. cold room into the 61.5° F. room all but four of the specimens showed expulsion, the highest number of spots for one slide being 52. On the second and third days there were no active ostioles and on the remaining 16 days very few in comparison with the first test of 17 days before being put in the room at 24° F.

(*e*) *Laboratory temperature, average 71.9° F.* All of the controls for the preceding and following experiments were run at laboratory temperature which averaged close to 72° F. At this temperature, as well as anywhere between 68° and 80° F., expulsion seems to occur very abundantly, whenever the proper moisture conditions are supplied. There does not appear to be any definite temperature which might be called the optimum or even any sharply defined limits. A few degrees one way or the other do not seem to make any appreciable difference. However, between the above stated temperatures, expulsion appears to take place most freely (see Table II).

(*f*) *Temperature 76.5°–80.5° F. Average 79° F.* The specimens were collected at Emilie, Pa., Nov. 5, 1913. The same methods were used as described above except that a Prairie State chicken incubator

was used to furnish the required temperature. 12 specimens were used for the experiment proper and 12 for the control. Two trays of specimens were put in the incubator on Nov. 6, 1913, 3 hours previous to wetting the bark and starting the experiment. This was done to equalize the temperature of all. A thermograph and a beaker of water for use in moistening the specimens were also kept in the incubator. The test was started at 3:00 P.M., on November 6 and continued for 9 days at an average temperature of 79° F. At this temperature the specimens seemed to shoot abundantly and just as actively as the control specimens. There was but little appreciable difference either in density or number of the spots; although the number of active ostioles per trap per day for all controls was 72.6, and 84.5 for the specimens at 79° F. (see Table II).

(*g*) *Temperature 82°–86.5° F. Average 84.6° F.* Specimens were collected at Emilie, Pa., Nov. 19, 1913, the customary methods used in their preparation, and the experiment started November 20. The same incubator was used as for the preceding (79°) experiment. The trays of specimens were put in the incubator at 3:30 P. M. in order to equalize the temperature but the bark was not drenched with water or slides adjusted until 10:00 A. M. The test was continued for 11 days, during which time all of the 12 specimens showed active ostioles but expulsion was rather moderate and could not be termed abundant except in some few individual cases on certain days. The control specimens shot abundantly. For the first eight days, while the amount of expulsion from the control specimens exceeded that from test specimens, it was more noticeable during the last three days when the effect of the high temperature began to be very evident. After the first day the density of the spots was always greatly in favor of the control. On the whole the amount of expulsion at 84.6° F. should be characterized as medium in comparison with the abundant expulsion at the more favorable temperature (see Table II).

(*h*) *Temperature 89°–92.5° F. Average 90.8° F.* Specimens were collected Oct. 23, 1913, at Emilie, Pa., and prepared the same as the preceding ones. 12 specimens were used for the experiment proper and 12 for the control. An incubator was used as in the two preceding tests, to obtain the desired temperature. The specimens were prepared on October 24 and kept moist until October 27 by wetting only the blotting paper. Absorption from the paper however furnished sufficient moisture in 17 out of the 24 specimens to cause considerable

spore expulsion. Two trays were put in the incubator at 11:00 A. M., on October 27, in order to equalize the temperature but the specimens were not wet until 5:00 P. M. the same day. The test was continued for 7 days in the incubator. On the first day at this temperature 8 traps showed expulsion averaging about 70 spots per trap. However, after the first day only three spots were noted and these from 2 slides on the last day. The abundant shooting on the first day in the incubator was probably due to the fact that the specimens had been shooting rather abundantly for two days previous to starting the experiment at this temperature. In comparison with the control which shot abundantly, there was a very light expulsion at 90.8° F. (see Table II).

(*i*) *Temperature 97°–100° F. Average 98.4° F.* The specimens were collected at Emilie, Bucks County, Pa., prepared by the same method as for the preceding tests, and the bark wet and experiment started April 2, 1913. 12 pieces of bark were used for the experiment proper, and 12 for the control. All of the specimens were kept at laboratory temperature for the first day and on April 3 half were put in the high temperature room and the others retained at the laboratory as controls. The test was continued for 15 days at an average temperature of 98.4° F. For the first 5 days the specimens were wet once a day but this was not sufficient because of drying out due to the high temperature. Only two active ostioles were recorded during this 5-day period and those from a single specimen on the fourth day. After the fifth day and until the 15th, when the test was brought to a close, the specimens were moistened twice a day and not allowed to dry out. On the sixth day 8 of the traps shot spores but expulsion was very light, there being only 41 spots from all. On the 7th and 8th days three specimens showed expulsion of spores but there were less than 20 spots each per day. For the remaining 7 days, there was absolutely no expulsion. The traps were then moved to the laboratory and kept under observation for 22 days. For the first 14 days there was practically no expulsion but after that they gradually picked up and on the last 3 days of the test every specimen shot rather freely. The control traps at laboratory temperature averaging 70.3° F. showed abundant expulsion, or an average of 64.6 active ostioles per specimen per day, while the specimens at 98.4° gave an average of only 0.4 (see Table II).

The results of the preceding tests on the relation of temperature to ascospore expulsion are summarized in Tables II and III.

Under artificial conditions in the laboratory expulsion of ascospores is entirely inhibited at low temperatures. With rise in temperature expulsion begins and activity of this process increases until finally expulsion of spores becomes abundant at temperatures ranging from about 68° to 80° F. At higher temperatures there is a gradual cessation of activity until at body temperature it is reduced to practically nil.

TABLE II

SUMMARY OF TESTS ON EFFECT OF TEMPERATURE ON ASCOSPORE EXPULSION

No. of Traps Used	No. of Days Tested	Temperature F.	Total No. of Spots Recorded	Av. No. of Active Ostioles per Trap per Day
21	13	38.6	0	0
23	13	72.5	14,424	48.2
20	14	53.8	408	1.4
19	14	72	6,627	24.9
12	10	56.25	1,503	12.5
12	10	70.8	12,969	108
12	17	62.5	3,609	17.7
12	17	72.8	16,871	82.7
12	9	79	9,125	84.5
12	9	72.2	8,950	82.8
12	11	84.6	4,232	32.06
12	11	72.2	9,664	73.2
12	7	90.8	462	5.5
12	7	72.5	8,112	96.5
12	15	98.4	74	0.4
12	15	70.3	11,643	64.6

It is interesting to note that there is a somewhat parallel relation between temperatures favorable for growth of the blight fungus and temperatures favorable for ascospore expulsion. The minimum, optimum and maximum temperatures for growth as given by Shear and Stevens (19) are as follows:

	Centigrade	Fahrenheit
Minimum	8–9	46–48
Optimum	18–28	64–82
Maximum	35	95

TABLE III

AMOUNT OF ASCOSPORE EXPULSION AT DIFFERENT TEMPERATURES

Specimen and Trap Numbers	Average Temperatures F.	No. of Active Ostioles per Trap per Day	Amount of Expulsion
11–18; 26–38	38.6	0	None
52–71	53.8	1.4	Very light
293–304	56.25	12.5	Light
150–161	62.5	17.7	Light
	71.9	72.6	Abundant
		Average for all controls	
245–256	79	84.5	Abundant
269–280	84.6	32.06	Medium
233–244	90.8	5.5	Very light
	98.4	0.4	Traces

It would appear from this comparison that continued ascospore expulsion is dependent to a certain extent upon growth. This is also substantiated by the tests made on the duration of ascospore expulsion as recorded in the following pages.

DURATION OF ASCOSPORE EXPULSION

Probably one of the most remarkable facts to be noted in regard to ascospore expulsion under artificial conditions is the power which the perithecial stromata have to expel spores for an almost indefinite period, provided they are given the proper temperature and moisture conditions.

On December 24, 1912, 23 specimens collected at Emilie, Pa., two days before, were prepared and a duration experiment started at laboratory temperature using the same method as for the temperature tests. Daily records were taken for 5 months and 17 days and at the end of this time, June 9, 1913, all but three of the specimens showed some active ostioles. One of these three specimens was overgrown by molds and undoubtedly had ceased shooting permanently but the other two, according to their former records, would probably have started up again. One peculiarity of ascospore expulsion is the fact that when first brought in from the field, the perithecia will generally shoot spasmodically for some days, that is, they will expel spores abundantly for one, or sometimes two or three days in succession, and then drop off to a very small amount. Then the succeeding days they will either gradually pick up and shoot abundantly for two

or three days or expel at their maximum capacity the first day after the rest period. The length of this spasmodic expulsion varies with the specimens used. Sometimes it continues for two or three weeks and sometimes for only a few days. At the end of this type of expulsion comes a rest period when the specimens may either cease entirely or reduce expulsion to a minimum. This may continue for a short or long time. In one case shooting ceased entirely for 7 weeks after the spasmodic period, and then gradually picked up and for over 7 weeks shot abundantly without missing a day. Table IV shows for each specimen the number of days expulsion occurred during the entire period, the number of days when there was no expulsion, the total number of spots recorded and the average number of active ostioles per day for the days when expulsion occurred.

The records summarized in this table certainly indicate an almost phenomenal power of ascospore production by the chestnut blight fungus. The short period of continuous ascospore expulsion recorded by Anderson and Babcock (2) was but a meager expression of a most persistent power. These tests under laboratory conditions seem to point to the probability that perithecial stromata under natural conditions in the field will not exhaust their power to produce and eject ascospores within the limits of a single season. Field tests in progress will shed definite light on this point. We may point to this remarkable power as one of the several factors that combine to make the chestnut blight fungus the most pernicious parasite that has ever invaded our forests.

Effect of Removal of Perithecial Necks

Three experiments were carried out at laboratory temperature to determine what effect the removal of the perithecial necks would have on the expulsion of ascospores. The tests were as follows:

Two specimens collected at Emilie on Dec. 22, 1912, were used, one for the experiment and one for the control. Both had the perithecial necks well developed; in fact one large piece of bark was broken into two parts so that the material would be in the same stage of development. On one specimen, every neck, with part of the stromatic tissue beneath, was removed with a sharp razor. The specimen was examined thoroughly with a strong lens to make certain that there were no necks remaining. On Dec. 24, 1912, at 10:00 A. M. both pieces of bark were wet and slides adjusted over them. On the 3d

day the specimen with the necks removed shot 1 spot whereas the control in the meantime had expelled 77 spots. Examination showed that the necks were reforming and from the third day on expulsion from the regenerated necks was as good as from the control.

TABLE IV

SUMMARY OF TESTS ON THE DURATION (168 DAYS) OF ASCOSPORE EXPULSION

No. of Trap	No. of Days on which Expulsion Occurred	No. of Days on which there was No Expulsion	Total No of Spots Recorded	Average Number of Active Ostioles per Day for Days when Expulsion Occurred
4	128	40	721	5.6
5	164	4	12,140	74.0
6	145	23	4,893	33.7
7	143	25	4,266	29.8
8	73	95[1]	1,721	23.6
9	148	20	5,742	38.8
10	153	15	11,203	73.2
19	154	14	9,834	63.9
20	126	42	3,196	25.4
21	147	21	9,574	65.1
22	166	2	24,018	144.7
23	168	0	18,079	107.6
24	139	29	1,906	13.7
25	154	14	10,004	65.0
39	154	14	5,979	38.8
40	165	3	16,363	99.2
41	153	15	3,848	25.2
42	152	16	20,323	133.7
43	156	12	9,533	61.1
44	149	19	7,344	49.3
45	154	14	9,295	60.4
46	146	22	9,313	63.8
47	108	60	10,232	94.7

On Dec. 28, 1912, the necks were removed on two more specimens. In one case the specimen with the necks removed shot after 4 days. In the other case the specimen with the necks removed did not shoot for 7 days when 50 spots were recorded while the check shot the 4th and 5th days with 10 and 18 spots respectively.

On Feb. 12, 1913, the necks on 4 more specimens were removed and the test started with a check for each. In three cases the control specimens showed expulsion before those from which the necks had been removed. The latter averaged 4 days before any expulsion occurred. In the 4th case both control and experiment proper shot on the 3d day.

While the number of tests is hardly sufficient to warrant a final

statement it seems highly probable that the structure of the perithecial necks has much to do with the conduction of the asci to the ostiole where the expulsion takes place. It has been pointed out by Anderson (3) in his study of the structure of the perithecium that the periphyses of the neck "act as so many little springs" and press the asci back. It may be added that the ascus is also held in position and any back movement prevented by the periphyses which spring back after its passage through the neck. This function of the periphyses would explain the results obtained for spore expulsion when the perithecial necks are removed. The time required for the necks to regenerate varied from 3 to 5 days.

Ascospore Expulsion from Inverted Perithecia

In considering ascospore expulsion one of the questions which very naturally presented itself was how the asci arrived at the end of the long neck. It was conceivable that detached asci might float to the top of the neck in the water with which it appears to be filled when expulsion is taking place. As a result of a study of the morphology of the perithecium Anderson (3) claims that the asci are forced to the ostiole as a result of expansion due to water absorption, and then that the supply is kept up by the growth and maturing of new crops.

In order to determine whether ascospore expulsion would take place when the necks were pointing downward, two tests were made in which the bark bearing the stromata was placed in an inverted position. These two tests were as follows:

On Jan. 11, 1913, a two-inch branch showing well-matured perithecial material, collected at Emilie, Pa., was soaked in tap water for 20 minutes with the necks inverted. At the end of this time an object slide was adjusted to the under surface of the branch, so as to be 2–3 mm. below the ostioles. The bark on the upper part of the branch was peeled off so that water could be injected between the wood and bark and be absorbed by the inverted perithecia below. The bark was kept moist and the test run for 8 days. Expulsion was light but was obtained on every day except one, the highest number of spots being 25.

On Feb. 13, 1913, 6 flat pieces of bark from larger branches were treated similarly to the above tests except that wet blotting paper was

kept on top of the specimens to insure sufficient moisture for expulsion. The test was continued for 11 days and shooting was recorded from all but one specimen. Expulsion was moderate except on two days from the same piece when the spots were very abundant. One peculiarity of the spore prints from inverted specimens is that the majority of the spots are not round and clearly defined as they usually are when the spores are shot upward (fig. 1). The spot is generally

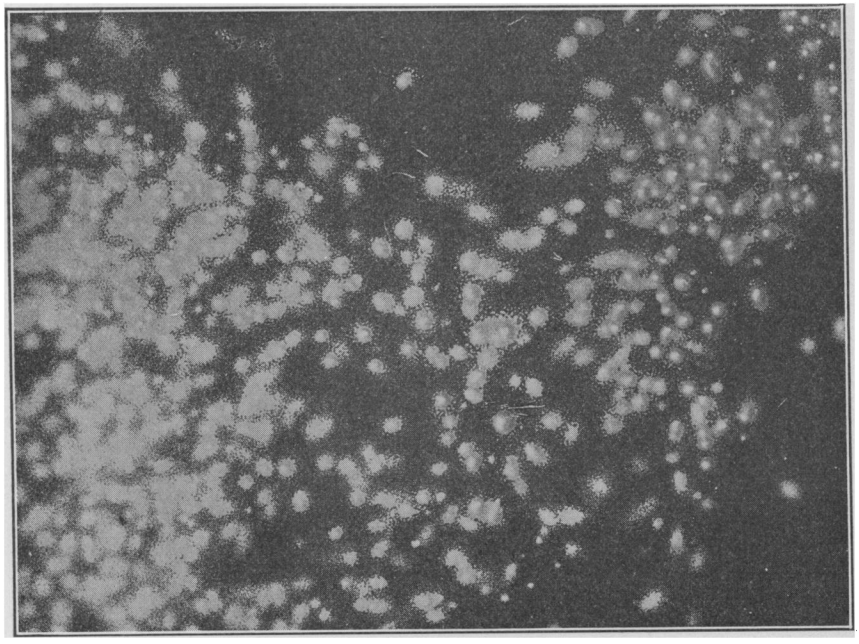

FIG. 1. A characteristic spore print of *Endothia parasitica* on an object slide, obtained when expulsion of the ascospores was taking place under optimum conditions.

elongated, denser at one end, and fades out at the other somewhat like a comet's tail (fig. 2). The explanation for these is probably the fact that many of the long perithecial necks are not in a vertical position but grow obliquely. When expulsion starts it is natural to suppose that the spores are shot out more forcibly and at that time a more or less distinct spot is formed. However, as shooting continues, the spores are ejected with less force and they fall short of the original spot and form the "comet's tail." When the necks point upward,

only a few "comet's tails" are formed and this is probably when the slide is of medium distance from the ostiole. Unless the slide is very close spores from obliquely directed necks never reach the slide.

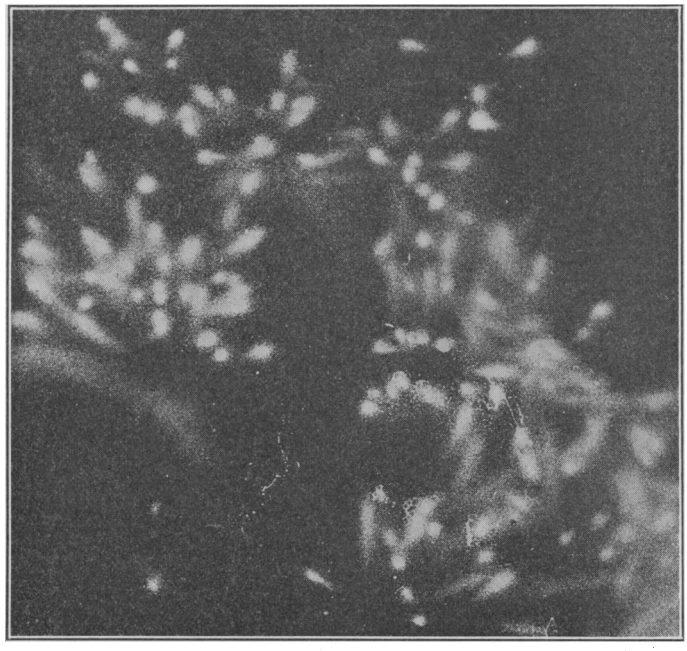

FIG. 2. Spore print of ascospores of *Endothia parasitica* obtained from perithecia in an inverted position. The comet-like spots are very characteristic.

THE EFFECT OF SATURATED ATMOSPHERE ON ASCOSPORE EXPULSION

In order to determine the effect of a saturated atmosphere upon ascospore expulsion, two tests were run at laboratory temperature. The specimens used were collected at Emilie, Pa. The experiments are as follows:

Three specimens of excellent perithecial material were selected for the test and two of them put in a large closed damp chamber on wet blotting paper while the third was kept as a check in an open chamber. The specimens were wet, slides adjusted and the experiment started Jan. 11, 1913. Records were taken for 27 days at the end of which time the control was found to have shot 71 spots to an average of 133 for the other two.

A more significant test because of the greater number of specimens was started Feb. 13, 1913, in damp chambers similar to those above. It was continued for 17 days with 6 specimens for the experiment proper and 6 for the control. At the end of the test it was found that while the checks showed a larger number of spots than the experiment specimens there was sufficient shooting from the latter to prove conclusively that expulsion will take place rather freely when the perithecia are in a saturated atmosphere.

This would point to ascospore expulsion as the result of high osmotic pressure from water absorption rather than to a drying effect from contact with the air as has been suggested by some writers (6). It should be pointed out in this connection that ascospore expulsion takes place during a rain under natural conditions, but continues for only a short period after the cessation of rain, the time varying with the humidity of the atmosphere.

Expulsion Without Direct Wetting

Two tests were conducted to determine whether ascospore expulsion could take place without direct application of water to the upper surface of the bark. They were as follows:

Specimens obtained from West Grove, Pa., were arranged on blotting paper in trays in the customary manner. Six were used for the experiment proper and six for the control. Water was applied every day to the blotting paper beneath the test specimens and care was taken not to give an excessive amount or to wet the upper surface of the bark, while the control specimens were drenched from above in the regular manner. The test was continued for 17 days at the end of which time all but one of the experiment specimens had expelled spores. Expulsion however was very light but the controls also showed but few active perithecia. While positive results were obtained they were not as conclusive as might be desired because the specimens were of poor quality. Practically all of them became contaminated with bacteria, and the perithecia appeared to be rather immature.

On Feb. 12, 1913, another test similar to the above was started using specimens from Emilie, Pa. Six traps were used for the experiment and six for the control. Daily records were taken for 16 days during which time there was some heavy ejection of spores from the

specimens which did not receive direct application of water. The control specimens however showed a still heavier expulsion. In all cases the water was applied to the blotting paper beneath the specimens and hence the only possible way by which it could reach the ostiole and cause expulsion was by absorption from below. This experiment was carried out mainly to determine whether ascospore expulsion would be likely to take place from stromata on logs or fallen limbs that might be left lying for some time in contact with the moist ground.

Expulsion from Isolated Pustules

A determination of the amount of expulsion from isolated pustules was attempted but the results obtained were not entirely satisfactory due principally to contaminated specimens. Two tests were conducted as follows:

Material obtained Jan. 18, 1913, from West Grove, Pa., was used and 16 pustules were isolated by cutting away all perithecial necks in the immediate vicinity. Then in order to make certain that no shooting would occur from regenerated necks, a mask was made of paper by cutting a hole, the same size as the pustule and slipping it over the latter. Nine of the 16 pustules became contaminated and gave no results. The remaining ones were kept under observation for 43 days. The number of necks to each pustule was counted and the maximum number of spots recorded on any one day is indicated in Table V.

Table V

Expulsion from Isolated Pustules

No. of necks per pustule	32	37	60	38	13	27
Maximum no. of spots obtained in one day	17	19	12	27	12	9
Percentage of active ostioles	53.1	59.3	20	71	92.3	33.3

A set of specimens similar to the above was started Feb. 15, 1913, and run at laboratory temperature for 41 days, except 4 pustules which were discontinued at the end of 22 days. The material was obtained at Emilie, Pa., on February 6. The same method was used as in the preceding test and 23 pustules were isolated. Table VI shows the results of this experiment.

It may be noted that pustules with necks ranging in number from 1–60 were used, and that activity from all ostioles was rare during

any one day. The length of time a single perithecium will continue to expel spores has not yet been determined.

Effect of Alternate Wetting and Drying upon Ascospore Expulsion

A test was started at laboratory temperature April 2, 1913, for the purpose of determining the effect of desiccation upon ascospore expulsion. Material from Emilie, Pa., was used and consisted of 5 sets of specimens with 3 to a set. No. 1 was wet and examined every day, No. 2 was wet every 2d day, No. 3 every 3d day, No. 4 every 5th day and No. 5 every 7th day.

The test was conducted for 42 days. The set which was allowed to dry one day gave by far the best expulsion. Shooting continued heavy to the end of the test and on the whole was considerably better than from the set which was wet every day. The sets wet every 3d, 5th, and 7th day respectively gave poor expulsion. One day of drying gives optimum results but thereafter the longer the period of desiccation the fewer the number of spots. The effect of alternate

Table VI

Expulsion from Isolated Pustules

No. of Necks per Pustule																						
7	5	7	5	5	7	7	60	1	37	5	47	24	47	15	19	19	19	29	34	19	23	18
Maximum No. of Spots Obtained in One Day																						
2	5	4	0	0	5	5	29	0	1	4	40	18	34	14	15	1	10	15	13	15	15	11
Percentage of Active Ostioles																						
28	100	57	0	0	71	71	48	0	3	80	85	75	72	93	79	5	53	52	38	79	65	61

wetting and drying is shown by Table VII.

Table VII

Effect of Alternate Wetting and Drying on Spore Expulsion

Time Moistened	Amount of Spore Expulsion
Every day	Abundant first week; moderate thereafter
Every 2d day	Heavy expulsion every day
Every 3d day	Light
Every 5th day	Very light
Every 7th day	Practically none

Field tests already carried out show that these results are no indicator of what may be expected under natural field conditions.

SUMMARY

1. Under artificial conditions in the laboratory spore expulsion is entirely inhibited at low temperatures. At higher temperatures there is a gradual increase in expulsion, with the optimum between 68° and 80° F. Spore expulsion was tested at temperatures ranging from 36° to 100° F. The results obtained in the laboratory substantiate the field results which have shown the cessation of spore expulsion during the winter period.

2. Perithecia show an almost phenomenal power of spore production, as shown by the fact that spores were expelled from some specimens every day for 168 days. Some perithecia were still active when the test was discontinued.

3. The necks of the perithecia play an important part in the mechanics of spore expulsion. Perithecia from which the necks have been removed cease to expel spores. Under favorable conditions for growth the necks will be regenerated, and with the formation of new necks spore expulsion is resumed.

4. Expulsion continues from inverted perithecia, the spore prints having a characteristic form. The asci are apparently brought to the ostiole as a result of pressure within the perithecium.

5. Spore expulsion will occur in a saturated atmosphere but appears to be more pronounced when specimens are permitted to dry out gradually.

6. Direct wetting of the stromata is not necessary for spore expulsion, but sufficient moisture may be absorbed from below. This points to the probability that fallen logs or bark bearing perithecial pustules may absorb sufficient moisture from the wet ground to cause spore expulsion.

7. In general all of the perithecia of a pustule are not expelling spores at the same time. The reverse is sometimes true for small pustules, but for larger pustules the maximum number of active ostioles varies from 30 to 90 per cent of the total.

8. Even under laboratory conditions, alternate wetting and drying does not inhibit the process of spore expulsion, but the best results are obtained from specimens moistened every other day.

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